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Technical Note

Tissue Distribution of Cisplatin After Hepatic Arterial Injection of a Cisplatin–Lipiodol Suspension Containing Phosphatidylcholine to Rabbits Carrying VX-2 Hepatic Carcinoma

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INTRODUCTION

Cisplatin (CDDP) has been used in the treatment of many solid cancers (1). Recently, it has been proposed for the treatment of hepatic carcinoma (2,3); however, its toxicity to kidney, blood, and neuron remained unsolved (4).

The oily lymphographic agent, Lipiodol (LPD), has been found to remain selectively in the hepatic tumor site for a long time after injection into the hepatic artery (5). Various formulations of anticancer agents coupled with LPD, i.e., solutions (6), emulsions (7), and suspensions (8), have been tested in the treatment of hepatic carcinoma by hepatic arterial administration.

We prepared suspensions composed of CDDP and LPD with varying phosphatidylcholine (PC) contents, as a dispersing agent, followed by the *in vitro* evaluation for the release of CDDP from the suspensions.

In order to clarify the effect of PC addition on the tissue distribution of CDDP, tissue concentrations of platinum (Pt) were also examined after injection of the suspensions into the hepatic artery of rabbits transplanted with VX-2 tumor in the liver.

MATERIALS AND METHODS

Materials

A saline solution of CDDP (CDDP-S, 0.5 mg/ml) and CDDP powder were kindly supplied by Nippon Kayaku Co.,Tokyo. LPD, an etyle ester of the fatty acid of poppyseed oil (38% iodine by weight) purchased from Kodama Co., Tokyo, was a product of Laboratorie Guerbert, Paris. Egg PC (Lot No. 65) was kindly supplied by Asahikasei Ind. Co., Ltd., Tokyo. Pt and nickel (Ni) standard solutions (1000 ppm, respectively) were obtained from Wako Pure Chemical Ind. Co., Osaka, Japan. All other chemicals were of special reagent grade.

Preparation of a Series of CDDP-LPD Suspensions Containing Different PC Contents

Various amounts of PC (0, 2, 20, 40, 60 mg) were gradually mixed with 1 ml of LPD in an agate mortar, respectively. Each mixture was collected in a syringe, followed by heating at 80°C for 5 min to obtain a transparent liquid. One milliliter of each transparent liquid was introduced into 20 mg of CDDP powder, mixing them again in an agate mortar. Resultant suspension was collected in a vial, followed by ultrasonication for up to 30 min, resulting in well-dispersed CDDP-LPD suspensions (CDDP-L-P). Each CDDP-L-P suspension was submitted to experiments immediately after preparation.

In Vitro Release Study

One milliliter of each CDDP-L-P suspension was placed in a dialysis tube (Visking Company; 8/32 in., 6.4-mm i.d.), and the tube was transferred to a cuvette containing 30 ml of an incubation medium (saline solution), followed by shaking at 37°C. At appropriate time periods, a 25-μl aliquot was taken from the incubation medium and injected into a high-performance liquid chromatography (HPLC) system with an ultraviolet detector. HPLC conditions were as follows: column, 4.6-mm-i.d. × 250-mm stainless-steel column packed with LiChrosorb NH_2 (10 μm, Merck); mobile phase, acetonitrile–saline (93:7); flow rate, 2 ml/min; wavelength, 210

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nm; and column temperature, 50°C. Typical retention time of CDDP was 4.0 min.

Animal Study

Male Japanese White rabbits, weighing 2.0 to 2.2 kg, were used. Rabbits were anesthetized by intravenous administration of sodium pentobarbital (25 mg/kg) before laparotomy. A 1-mm^3 cube of VX-2 tumor, maintained by successive transplantation into the liver of rabbits, was transplanted into the subcapsular parenchyma of the left anterior lobe of the liver. After confirming the tumor size growing up to about 10–20 mm in diameter for 14 days, 20 mg/ml of CDDP-L-P_3 (a content ratio of CDDP to PC of 1:3), the same concentration of CDDP-L (the suspension without PC) and 0.5 mg/ml of CDDP-S (commercial product) were injected into the left proper hepatic artery using the injection device, which consisted of a polyethylene tube connected to a 27-gauge needle on one end and a 0.5-ml (for CDDP-L-P_3 and CDDP-L) or a 10-ml (for CDDP-S) syringe on the other end, in a volume of 0.1, 0.1, and 4 ml/kg, respectively. The dose of CDDP administered was 2 mg/kg, in common with CDDP-L-P_3, CDDP-L, and CDDP-S, and the injection time was 30 to 50 sec.

Three-milliliter blood samples were collected from the ear vein at various times (0.25–48 hr) after injection and plasma was separated by centrifugation, followed by detection of plasma Pt concentrations. Tissue samples of liver, kidney, bile cyst, spleen, lung, heart, muscle in the thigh, and brain were excised at 48 hr after hepatic arterial injection of each preparation. In particular, the liver specimen was further divided into three parts: the tumor site, the nontumor site adjacent to the tumor in the left anterior lobe (the near site), and the nontumor site distant from the tumor in the right anterior lobe (the far site).

Pt contents in plasmas and tissues were determined by flameless atomic absorption spectrophotometer (Hitachi Z-8000, Hitachi Ltd., Tokyo) using Ni as an internal standard. The values of Pt determined were represented as the values of CDDP.

RESULTS AND DISCUSSION

Figure 1 indicates the effect of PC on the release of CDDP from five kinds of CDDP-L-P suspensions. The release of CDDP gradually decreased with increasing PC content. In the case of CDDP-L-P_3, the release of CDDP was about one-third that of CDDP-L at 24 hr after starting the dialysis experiment. PC was found to modify the sustained release of CDDP from the CDDP-L-P suspension *in vitro*. Afterward, CDDP-S, CDDP-L, and CDDP-L-P_3 were submitted to the following *in vivo* experiments.

Pt tissue concentrations at 48 hr after hepatic arterial injection of 2 mg/kg of CDDP-S, CDDP-L, and CDDP-L-P_3 to rabbits transplanted with VX-2 tumor are shown in Table I. The hepatic arterial injection of CDDP-L-P_3 produced a tissue-selective distribution of Pt in comparison with CDDP-L or CDDP-S. The target tumor concentration of Pt after injection of CDDP-L-P_3 was 13.9 μg/g wet wt. The value was 1.9 times higher than obtained after CDDP-L and 5.3 times higher than after CDDP-S. Similarly, the Pt concentration in the nontumor site adjacent to the tumor (the near site) after

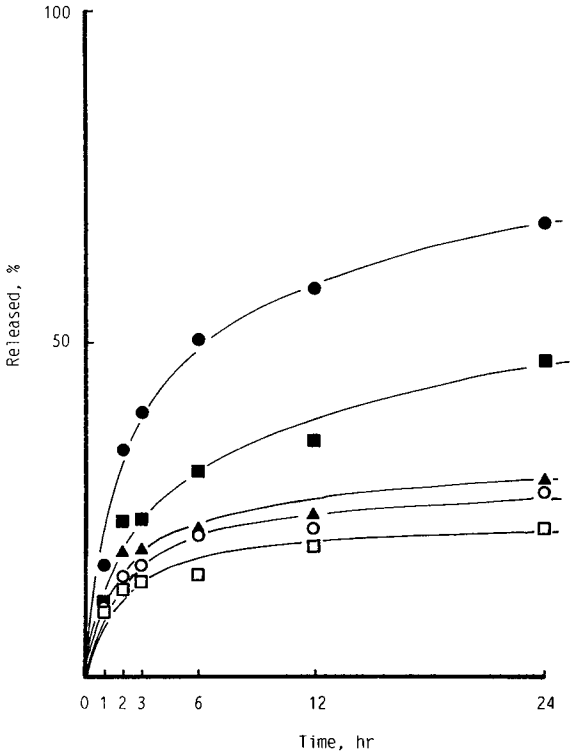

Fig. 1. Effect of PC on release of CDDP from its various CDDP-L-P suspensions. All suspensions contain 20 mg CDDP. PC content is as follows: ●, 0 mg; ■, 2 mg; ▲, 20 mg; ○, 40 mg; □, 60 mg. Each point represents the mean of at least three experiments.

injection of CDDP-L-P_3 was higher than that after injection of CDDP-L or CDDP-S. On the contrary, CDDP-L-P_3 resulted in a significantly decreased delivery to nontarget tissues, i.e., kidney and heart.

The higher localization of CDDP after administration of CDDP-L-P_3 in both the tumor site and the near site may be due to PC acting as a modulator for the sustained release of CDDP from the CDDP-L-P suspension, as observed in the *in vitro* experiment. The sustained release of CDDP by PC was reflected in the lower Pt plasma concentration curves (Fig. 2). The areas under the plasma concentration–time curves were reduced by 75% for total Pt following hepatic arterial injection of CDDP-L-P_3 as compared to CDDP-L.

For clinical use, hepatic arterial administration of CDDP-S was applied to the treatment of hepatic carcinoma in order to localize CDDP in the tumor (2,3). However, after intrahepatic arterial application CDDP also accumulates in the kidney as a major target of drug toxicity (Table I).

LPD was first used with the anticancer agent, stylene maleic acid neocarzinostatin (SMANCS), a lipophilic anticancer derivative of neocarzinostatin (6). Since then, several lipophilic prodrugs of anticancer agents have been synthesized for increased solubility in LPD (9,10). In the present study, we demonstrate that CDDP can be selectively delivered to the tumor site by administration in CDDP-L-P_3.

The mechanism of the selective accumulation of CDDP in the hepatic tumor site following hepatic arterial injection of CDDP-L-P_3 is not fully understood. We speculate that CDDP-L-P_3 gives rise to a mild ischemia in the hepatic ar-

Table I. Pt Concentrations in Various Tissues at 48 hr After Hepatic Arterial Administration of CDDP-S, CDDP-L, and CDDP-L-P_3 to Rabbits Transplanted with VX-2 Tumor[a]

Tissue	Pt (μg/g or μg/ml)		
	CDDP-S	CDDP-L	CDDP-L-P_3
Plasma	0.26 ± 0.06	0.10 ± 0.10	0.10 ± 0.03*
Brain	ND[b]	ND	ND
Muscle	0.09 ± 0.04	0.06 ± 0.05	0.03 ± 0.03
Heart	0.36 ± 0.04	0.13 ± 0.06**	0.09 ± 0.04**
Lung	0.41 ± 0.14	0.21 ± 0.09	0.12 ± 0.02
Spleen	1.05 ± 0.33	0.32 ± 0.02	0.31 ± 0.05
Bile cyst	0.81 ± 0.13	1.46 ± 0.59	1.04 ± 0.79
Kidney	4.07 ± 0.32	2.02 ± 0.28**	1.91 ± 0.40**
Liver			
Tumor	2.64 ± 0.53	7.27 ± 1.24**	13.9 ± 1.5***,****
Near site[c]	2.35 ± 0.55	6.27 ± 1.31**	9.02 ± 3.37
Far site[d]	1.58 ± 0.35	1.21 ± 0.34	0.63 ± 0.06

[a] Animals received a 2-mg/kg dose of CDDP. Each value represents the mean ± SD of three animals.
[b] Not detected.
[c] The nontumor site adjacent to the tumor in the left anterior lobe.
[d] The nontumor site distant from the tumor in the right anterior lobe.
* Significantly different from CDDP-S at $P < 0.05$.
** Significantly different from CDDP-S at $P < 0.01$.
*** Significantly different from CDDP-S at $P < 0.001$.
**** Significantly different from CDDP-L at $P < 0.01$.

tery, followed by sustained release of CDDP in the hepatic tumor site owing to PC, resulting in a site-selective localization of CDDP. Recently, chemoembolization for CDDP has been studied using a variety of embolic materials, i.e., gelatin sponge, and microcapsules (3,11,12). Chemoembolization therapy was considered advantageous at first because it prolonged the residence time of the anticancer agent within the tumor site. However, therapy is now limited to small tumors because collateral vessels develop within 2 or 3 weeks and occlusion in uneffected organs also occurs. The blood supply of the tumor in the liver is principally arterial, while hepatocytes are also bathed by portal blood flow (13). Therefore, the mild embolization by CDDP-L-P_3 in the hepatic artery may overcome the disadvantage of conventional chemoembolization.

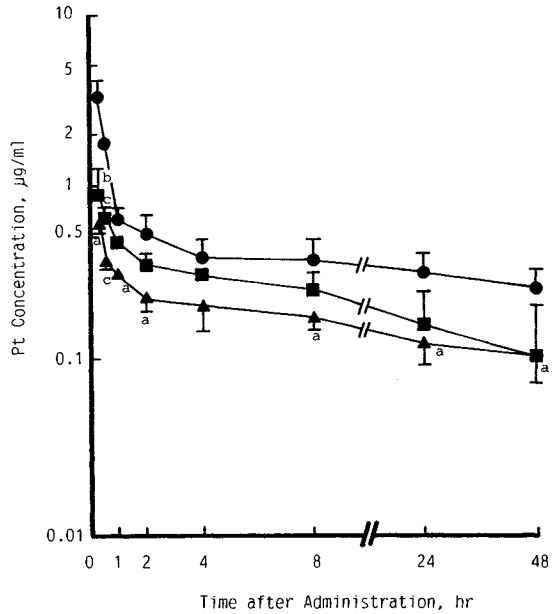

Fig. 2. Plasma Pt concentration curves after hepatic arterial administration of CDDP-S (●), CDDP-L (■), and CDDP-L-P_3 (▲) to rabbits transplanted with VX-2 tumor. Each point represents the mean ± SD of three animals. Significant differences from CDDP-S were assessed by Student's *t* test: (a) $P < 0.05$; (b) $P < 0.01$; (c) $P < 0.001$.

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